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Hydrology and Earth System Sciences Discussions

Interactive comment on "Vegetation composition and soil microbial community structural changes along a wetland hydrological gradient" *by* W. K. Balasooriya et al.

W. K. Balasooriya et al.

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Answer to the Referee #2

General comments

Comments: This paper deals with an interesting methodological approach for investigating the changes of vegetation composition and soil microcosm under a hydrological gradient in a natural wetland ecosystem. The final objective is to point out the relationships among the hydrological fluctuations, the distribution of vegetation compositions and the structural differences of the microbial community. In spite of the complexity of the treated matter, it is a pleasured to read this manuscript for its clearness and

comprehensibility. The scientific language used, the correctness of formulae and abbreviations contribute to the understanding of the research progress and outcomes. The description of experimental methodologies used is complete, Authors give an authentic possibility of reproducing each phase of the investigation. The methods used are not innovative in themselves; as Authors made it clear in the introduction section, stable isotope probing of phospholipid fatty acids in soil and plants has been widely used in previous works cited as well as 13CO2 pulse labeling of growing plants. The novelty of the scientific approach is based on the contemporary utilisation of the best technologies and analytical methods available for obtaining the more rigorous results in the contest of an intelligent research idea, which is relevant for the scientific concern of HESS.

Response: We would like to thank both reviewers for approving our work and for complimenting on the quality of our submitted manuscript. We found their suggestions very useful and have undertaken the revision of the manuscript to include most of them.

Specific comments

Comments: Title reflects the contents of the paper. Abstract is well written. References are appropriate. Conclusions are suitable in relation to results and discussion.

Response: Thank you

Comments: Some remark about the absence of detailed description of several methods used during the work. These descriptions can be incorporated in the text of the manuscript or can be given as enclosed materials (annexed technical notes):

Response: We incorporated these descriptions in the text as mentioned below.

Comments: p. 3875, line 24: please, describe method and materials used for CO2 injections;

Response: The following text is now included in the manuscript (pg 3875, line 24): "A Tedlar gas sampling bag (1L, Alltech) fixed to a 50ml gas sampling bulb with a stop

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cock was used in the field to temporarily store 13CO2 gas from the pressurized gas bottle, and allowed sampling of a known volume of 13CO2 gas at atmospheric pressure prior to injection into the labeling chamber. When the CO2 level in the chambers fell below 250 ppm, 100ml of 13CO2 was transferred into a 100ml gas tight syringe (1100, Hamilton) from the gas sampling bulb and injected into the labeling chamber through a septum. The injections were repeated four times more, approximately in one hour intervals."

Comments: p. 3876, line 24: please, briefly describe analytical and instrumental conditions for spectrometric measurements;

Response: The following text is now included in the manuscript (pg 3876, ln 22): "A subsample of ground shoot and root samples (1.5mg) and soil samples (20mg) were analyzed for C, N and 13C content using an Elemental Analyzer (EA) (ANCA-SL, Europa PDZ, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, Sercon, UK) (EA-IRMS). Samples measured in tin capsules were loaded into the EA using an auto sampler. The temperature at the combustion and reduction stages was 1800°C and 600°C respectively. Water is removed by a magnesiumperchlorate trap. Before entering the mass spectrometer, the gas stream passes a gas chromatograph which separates CO2 from N2 gas. In the IRMS (90 eV electron voltage, 144 μA trap, He carrier gas, continuous flow, 10-7 mbar vacuum pressure, 90% focus) gaseous molecules are ionized in the ion source, then separated according to their mass-to charge ratio and finally collected in an array of Faraday cups (three faraday cup collectors for m/z 44, 45, and 46). Reference CO2 of known isotopic composition was used for sample calibration and introduced directly into the source three times at the start and end of each run. Each sample was run in duplicate to ensure reliable mean 13C values."

Comments: p. 3877, lines 12-25, specify dimensions of silica gel columns, how much chloroform and acetone was used for elution, how much methanol for recovery of lipids;

Response: The following information is now given in the methods section: pg 3877, ln

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12 "Silica gel columns (CHROMABOND SiOH 500mg)", pg 3876, ln 13 "6 ml chloroform", "10 ml acetone", pg 3876, ln 14 "5 ml methanol"

Comments: please, describe better the method of transesterification (concentration of KOH in the final reaction mixture);

Response: We now describe the method of transesterification as follows (pg 3877, ln 14): "Phospholipids were eluted with 5 ml methanol and the solution was dried under N2. Phospholipids were subsequently transesterified by mild methanolysis (1:1 methanol-toluene, 0.2 M methanolic KOH, heated for 15 min at 35°C) to form volatilizable fatty acid methyl esters (FAMEs)."

Comments: specify operative conditions of spectrometer.

Response: The following text is now included in the manuscript (pg 3877, ln 16): "FAMEs were analyzed by capillary gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS) using a GC coupled to an IRMS via a combustion III interface (ThermoFinnigan DELTAPLUS XP). The samples were injected with an auto sampler (10-μL syringe with 70 mm needle) (ThermoFinnigan DELTAPLUS XP) into the GC (splitless mode, He carrier gas, constant He flow of 1 ml min-1, backflush at 1200 s, CP SIL column (100m Œ 0.25mm i.d.Œ 0.2μm film thickness; Varian Inc.)). The injection volume was 2 μL and the injector temperature was 250°C. The oven temperature was programmed at 75°C for 2 min., followed by a ramp at 5°C min-1 to 180°C with a 20 min. hold, and a final ramp at 2°C min-1 to 225°C with a 20 min. hold. The temperature of the combustion and reduction oven was 940 and 630°C respectively. Water removal was via a Nafion membrane. The IRMS (He carrier gas, continuous flow) consists of an open split inlet and operated at 142 eV electron voltage and 0.7 mA trap. Other conditions were as described above."

Technical corrections

Comments: "Technical corrections" p. 3875, line 23: Change 8220;plexi glass8221; in

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Plexiglas; p.3876, line 2: Change 8220;monitered8221; in monitored; , line 16: Change 8220;solution8221;in suspension; p. 3881, line 14: Table 3 has been cited first time before Table 2 (p. 3882, line 10); p. 3883, line 18: 8220;Could be shadowed8221;, better: could have been shaded (or shadowed); p. 3890, line15: 8220;realtive8221; in relative; line 25 8220;favors8221; in favor (see also p. 3892, line 15). Fig. 2: pay attention to the color of lines in the inset Response: All these changes were made in the revised manuscript.

Interactive comment on Hydrol. Earth Syst. Sci. Discuss., 4, 3869, 2007.